Development 125, 151-160 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV1241

REVIEW ARTICLE

Somite number and vertebrate evolution

Michael K. Richardson[1,*], Steven P. Allen[1], Glenda M. Wright[2], Albert Raynaud[3] and James Hanken[4]

[1]Department of Anatomy and Developmental Biology, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK
[2]Department of Anatomy and Physiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, C1A 4P3, Canada
[3]Laboratoire de Zoologie, Université Paul Sabatier, 118 Route de Narbonne, F 31062, Toulouse Cedex, France
[4]Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, CO 80309-0334, USA
*Author for correspondence (e-mail: m.richardson@sghms.ac.uk)

Accepted 11 November 1997; published on WWW 17 December 1997

SUMMARY

Variation in segment number is an important but neglected feature of vertebrate evolution. Some vertebrates have as few as six trunk vertebrae, while others have hundreds. We examine this phenomenon in relation to recent models of evolution and development. Surprisingly, differences in vertebral number are foreshadowed by different somite counts at the tailbud stage, thought to be a highly conserved (phylotypic) stage. Somite number therefore violates the 'developmental hourglass' model. We argue that this is because somitogenesis shows uncoupling or dissociation from the conserved positional field encoded by genes of the zootype.

Several other systems show this kind of dissociation, including limbs and feathers. *Bmp-7* expression patterns demonstrate dissociation in the chick pharyngeal arches. This makes it difficult to recognise a common stage of pharyngeal development or 'pharyngula' in all species. Rhombomere number is more stable during evolution than somite number, possibly because segmentation and positional specification in the hindbrain are relatively interdependent. Although developmental mechanisms are strongly conserved, dissociation allows at least some major evolutionary changes to be generated in phylotypic stages.

Key words: Somite number, Segmentation, *Hox* genes, Evolution, Phylotypic stage, Vertebrate

EVOLUTIONARY CHANGES IN SOMITE NUMBER

The vertebrate body plan is made up of repeating and non-repeating patterns of organ primordia (Cooke, 1975). Repeating patterns include somites, feathers, pharyngeal arches, rhombomeres and, less obviously, the digits. At least two mechanisms are involved in generating repeating patterns: a segmentation mechanism and, superimposed on this, a positional field for making the segments or units different (Ingham and Martinez Arias, 1992). Raff has pointed out that it is relatively easy for evolution to vary the total number of segments in some repeating series (Raff, 1996). A striking example of this is the variation in the number of body segments in vertebrates (Fig. 1).

Vertebrae develop from the sclerotomes of the somites, which themselves segregate from mesoderm laid down during gastrulation and tail development (Verbout, 1985; Keynes and Stern, 1988; Christ and Wilting, 1992). Our aim in this article is to assess how evolutionary changes in somite number relate to modern concepts of evolution and development such as the developmental hourglass, the phylotypic stage and the zootype. Developmental mechanisms controlling somite number are discussed. We argue that uncoupling or dissociation between repeating patterns and positional fields, as exemplified by somite development, is an important feature of development and evolution in a number of systems.

Vertebrate segment number: comparisons with invertebrates

Only vertebrates and other chordates have a tailbud. After gastrulation, the vertebrate tailbud can continue to make somites (Gont et al., 1993; Tucker and Slack, 1995). Vertebrate segmentation is therefore, in principle, an open-ended system. This may help explain how total somite number can vary widely between vertebrate species. Somitogenesis in vertebrates is analogous to the process of segment formation in many annelids and arthropods, including 'short germ' insects, where segments are added from a growth zone that lies in front of the anal segment or telson (Anderson, 1973; Nagy and Carroll, 1994).

In some of these lineages, the number of segments is indefinite and varies between individuals. In many other groups of invertebrates, the final number of segments in the adult body

is invariant. Some precise, but as yet unknown, mechanism must terminate segment formation. For example, in leeches, an indefinite number of segment precursor cells is budded from the teloblasts, but only 32 segments differentiate; supernumerary cells die or fuse with the yolk syncytium (Weisblat et al., 1988).

As may be the case in the backbone of vertebrates, segment differentiation in centipedes is more consistent with position along the body than with segment number (Minelli, 1992). Segmentation is best understood in *Drosophila*, but this model system provides little insight into the general problem of segment numbers. *Drosophila* and other 'long-germ' insects have no posterior growth zone. Segments are generated almost simultaneously by the subdivision of an existing field of cells, using a cascade of patterning interactions that are triggered by maternal gradients (Pankratz and Jackle, 1993). Growth and segmentation are uncoupled. This mechanism – which is presumably highly derived – has more in common with the segregation of rhombomeres in the vertebrate embryo than with somitogenesis from a blastema.

Vertebrates show extreme variations in segment number

In cartilaginous fishes such as sharks, there may be several hundred vertebrae (Springer and Garrick, 1964). In teleosts, a typical vertebral number is 48, although the range is very wide (Baer, 1964). Long-bodied teleosts such as eels may have as many as 200 vertebrae. At the other extreme the platyfish has around 6 trunk and 20 tail segments (Tavolga, 1949). Like teleosts, amphibians also show wide variations. Adult frogs have 6-9 presacral vertebrae, the lowest count of any terrestrial vertebrate (Young, 1962). By contrast the caecilian amphibians have worm-like bodies and 95-285 trunk vertebrae (Duellman and Trueb, 1994). A cautious estimate, from incomplete fossil data, is that when some of these long-bodied vertebrates evolve, they show an average increase of one vertebra every million years (R. Carroll, personal communication).

The highest vertebral numbers are found in some reptiles. Snakes may have hundreds of vertebrae – as many as 565 in the extinct *Archaeophis*. In modern birds, vertebral number is more stable, a typical range being 37-53 (Starck, 1996). In mammals, the number of precaudal vertebrae is also relatively stable, but the number of vertebrae in the tail varies from 3 to 47 (Baer, 1964; Anderson and Jones, 1984). Interestingly, vertebral number is much more plastic in some dolphins (genus *Phocoenoides*) than it is in terrestrial mammals, and may approach 100 (Anderson and Jones, 1984).

DEVELOPMENTAL CONTROL OF SOMITE NUMBER

In this section, we discuss developmental mechanisms that might be targets for evolutionary changes in somite number.

Vertebrate segmentation genes

In insects, body segmentation and segment identity are controlled by different genes (Ingham and Martinez Arias, 1992; Lawrence, 1992). The same appears to be true for the vertebral column. Although vertebrate segmentation genes have proved elusive, several promising candidate genes have recently been identified. Unfortunately, we are a long way from understanding the relation of these genes to the control of somite number. In the mouse, the Eph-related receptor tyrosine kinase *Sek-1* is expressed in two stripes in the unsegmented mesoderm immediately caudal to the last-formed somite (Nieto et al., 1992). The first narrow stripe may correspond to the anterior half of the next somite to form, and a broader stripe caudal to that may represent the next but one somite. Expression patterns which appear before segments have formed, and which correspond with the future position of segments, are known as isomorphic prepatterns (Wolpert and Stein, 1984). While these patterns are suggestive of segmentation genes, functional studies are needed for a definitive answer.

Signalling by homologues of *Drosophila Delta*, and its receptor *Notch*, is important for normal somite development in vertebrates. Targeted disruption of *Notch-1* leads to defective somitogenesis (Conlon et al., 1995) as does disruption of *RBP-Jκ*, a transcription factor in the *Notch* signalling pathway (de la Pompa et al., 1997). The correct expression of *Notch-1*, and the mouse *Delta* homologue *Dll1*, is dependent on the transmembrane protein presenilin 1 (Wong et al., 1997). In *Xenopus*, *X-Delta-2* is expressed in a segmental pattern in the paraxial mesoderm that precedes overt somite formation, suggesting a role as a segmentation gene. However, targeted disruption shows that, in mice, the *Delta* homologue *Dll1* is probably involved in the maintenance of somite borders rather than in the establishment of the primary segmental pattern (Hrabe de Angelis et al., 1997).

her1, the zebrafish homologue of the *Drosophila* pair-rule gene *hairy*, is expressed in alternating somites (Müller et al., 1996). Homologues of the *Drosophila* segment-polarity gene *engrailed* may be involved in the formation of enterocoelous somites, such as the first 8 segments of amphioxus (Holland et al., 1997) and the mandibular head cavity of the lamprey, *Lampetra japonica* (Holland et al., 1993; De Robertis, 1997). This and other evidence suggests a common evolutionary origin of segmentation in vertebrates and at least some invertebrate taxa (De Robertis, 1997).

Other putative segmentation genes have been identified in the vertebrate hindbrain. The zinc-finger genes *kreisler/valentino* and *Krox-20* are important for normal segmental patterning of rhombomeres (reviewed by Lumsden and Krumlauf, 1996). However, in the *Krox-20* null mutant mouse, although the normal pattern of rhombomeres is disrupted, the underlying segmentation of the hindbrain is maintained (Schneider-Maunoury et al., 1997). *Sek* genes are also likely to have a role in hindbrain segmentation (Irving et al., 1996). Disruption of *Sek-1* signalling by injection of a tk^- truncated RNA disrupts hindbrain segmentation in zebrafish and *Xenopus* embryos (Xu et al., 1995).

Axial growth and somite number

Tam (1981) proposed a relationship between the rate of axial growth, the rate at which somites are laid down and the size of somites. Thus tail somites in the mouse are small and numerous, and the rate of axial elongation when they are laid down is relatively slow. By contrast, the lumbar and sacral somites are larger and fewer, and are segregated at a time of rapid axial elongation. Thus, in the mouse, there may be a negative correlation between the rate of axial elongation, and somite number. Other species appear to show a positive correlation.

Caecilian embryos undergo rapid elongation during neurula stages. As the body lengthens, large numbers of somites are

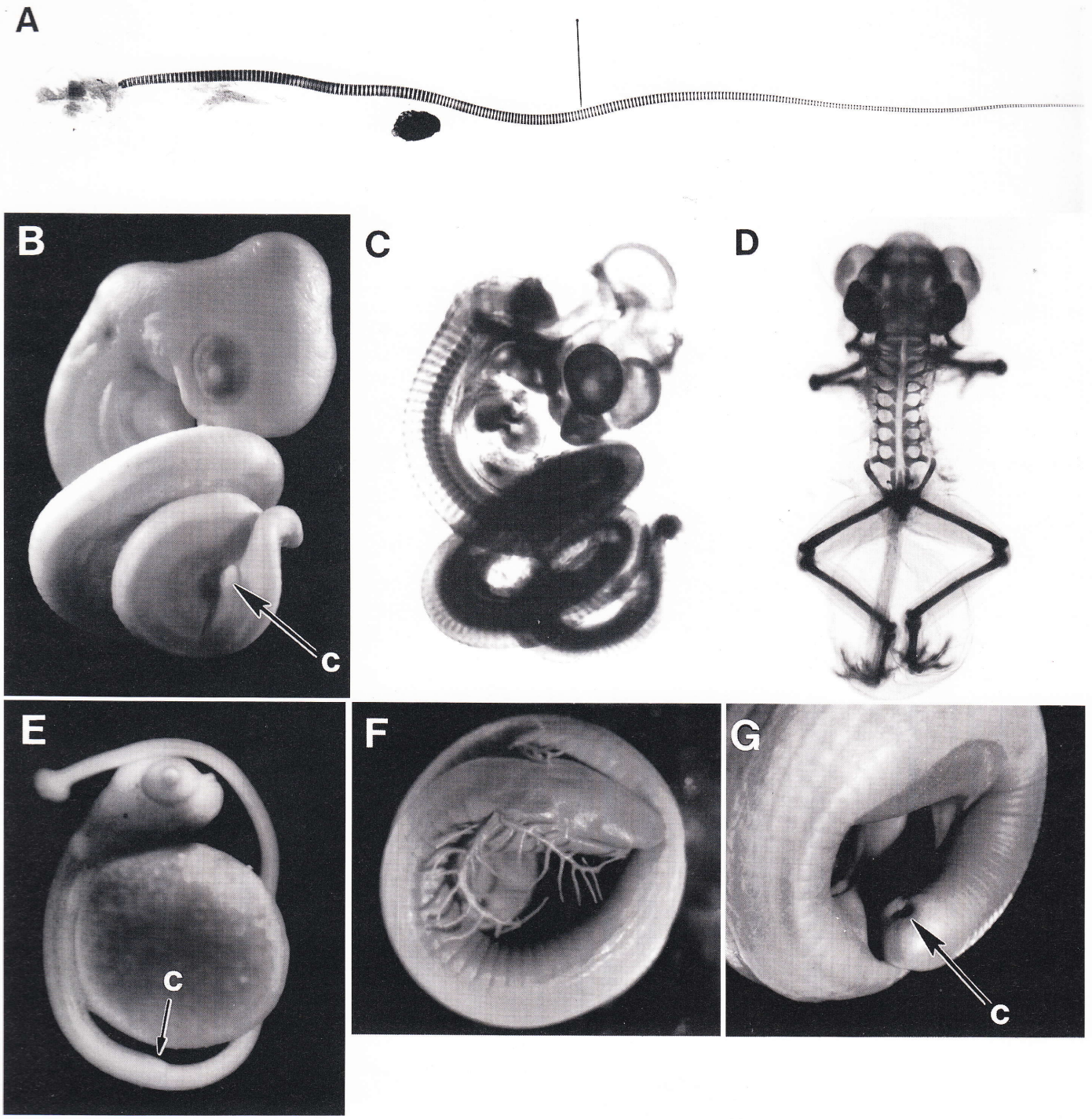

Fig. 1. Different patterns of segmentation in long-bodied and short-bodied vertebrates (postphylotypic stages). (A) Radiograph of immature thresher shark, *Alopias vulpinus*. Needle indicates position of cloaca. Note the very large number of body segments. Reproduced from Springer and Garrick (1964). Courtesy Dr V. Springer, Smithsonian Institution, NMNH, Division of Fishes. (B) Snake, *Natrix maura*, 5½ days after laying (from the lab of A. Raynaud). *c*, position of the cloaca. (C) As B, but stained with Alcian blue and cleared. Note the large number of precloacal body segments, represented by vertebrae in early stages of chondrification. (D) Puerto Rican treefrog, *Eleutherodactylus coqui*, stage 14 (Townsend and Stewart, 1985), dorsal view (from the lab of J. Hanken). Stained with Alcian blue and cleared. Note the low number of body segments. Somites were present in the tail at an earlier stage but have been resorbed. (E) Greater pipe-fish, *Syngnathus acus*, pectoral fin bud stage, showing a highly elongated body axis. Dorsal view (Courtesy Netherlands Institute for Developmental Biology). *c*, position of the cloaca. Unlike the caecilian (F,G), the caudal vertebrae in the pipefish make a substantial contribution to total segment number. (F) Larva of caecilian amphibian, *Idiocranium* sp., branched external gill stage (Courtesy Netherlands Institute for Developmental Biology). Dorsal view showing the highly elongate body. (G) Detail of F showing the cloaca *(c)* situated almost at the terminal point of the body. The tail region in adult caecilians makes no significant contribution to the total vertebral count.

segregated (Sammouri et al., 1990; Duellman and Trueb, 1994). The hagfish (a chordate) also has a highly elongated primary axis and forms a high somite number: 75 by the tailbud stage (Fig. 2). Recent studies on reptiles support the idea of a positive correlation between axial elongation and somite number. Raynaud (1994) examined somitogenesis in the 'slow worm' *Anguis fragilis* and the green lizard *Lacerta viridis*. The slow worm is a limbless lizard with a long body, rather like a snake. The lizard, by contrast, has well-developed limbs and does not have a highly elongated body. When the embryos were compared, it was found that the slow-worm embryo elongated, and laid down somites, more rapidly than the lizard.

It is not clear whether an increase in body length is primary or secondary to an increase in somite number. In other words, does somite number regulate according to the length of the strip of presomitic mesoderm? Experiments indicate that this may not be the case (reviewed in Tam, 1981). For example, if *Xenopus* embryos are made smaller by ablating tissue at early stages, the same number of somites is formed, but the somites are smaller (Cooke, 1975). These conclusions were reached by comparing operated embryos with stage-matched controls. It will be interesting to determine whether these findings are applicable to other amphibians, such as the direct-developing frogs, which show different patterns of development to *Xenopus* (Elinson, 1987).

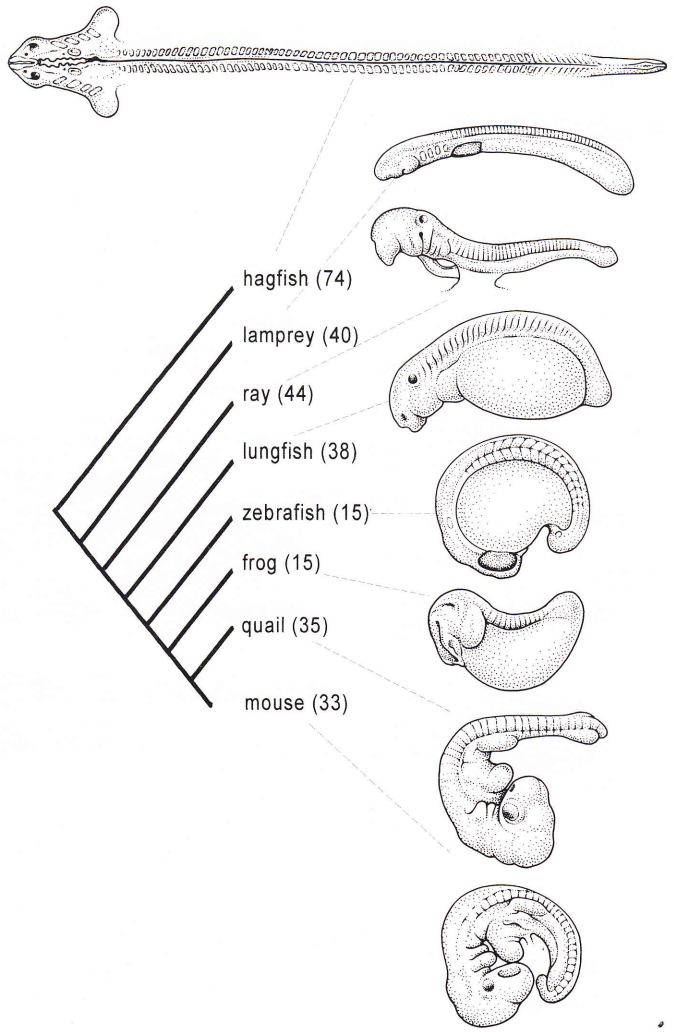

Fig. 2. Vertebrate embryos at the phylotypic (tailbud) stage show wide variations in somite number (parentheses). The hagfish (redrawn from Dean, 1899) is included although it probably belongs to a separate taxon within the chordates. For other embryos see photos and references in Richardson et al., 1997. All left lateral views except the hagfish, which is dorsal. (Not to scale).

Cell cycle of somite precursors

Experimental data support a model in which somite number is controlled in part by species-specific cyclical properties of somite precursor cells (Cooke, 1981). Using clonal analysis, Stern and colleagues have shown that precursors in the mesoderm of the chicken embryo give rise to progeny in every 5-7th somite (Selleck and Stern, 1991; Stern et al., 1992). Heat shock of chick embryos produces defects in every 6-7th somite, a periodicity that corresponds quite well with the predicted cell cycle time of somite precursors (Primmett et al., 1988, 1989; Stern et al., 1988). Heat shock in anuran and zebrafish embryos also gives rise to defects in the somite series, but these are single rather than repeated (reviewed by Keynes and Stern, 1988; Kimmel et al., 1988).

It would be interesting to determine whether somite precursors show different cell-cycle times in species with different somite numbers. For example, do snake somite precursors have a very short cell cycle? Tam (1981) has drawn attention to the possible parallels between cell cycle time in somitogenesis and skeletogenesis in the limb. According to the progress zone model, there is a relationship between the number of skeletal elements laid down along the proximodistal axis of the limb and the number of cell cycles completed by mesoderm cells in the progress zone (Summerbell et al., 1975). Whether or not the tailbud behaves in this way remains to be determined.

Other factors

While differences in segment number among species are due principally to evolutionary changes in somitogenesis, other factors can be involved. The low vertebral number in adult frogs is due to fusion of vertebrae, and the loss of the tail at metamorphosis (Duellman and Trueb, 1994). Although anurans such as *Xenopus* have only 6-9 presacral vertebrae as adults, more than 40 somites are segregated in the *Xenopus* embryo (Nieuwkoop and Faber, 1967). Tucker and Slack (1995) have shown that the trunk-tail boundary slides anteriorly along the primary axis during *Xenopus* development. We suggest that this may contribute to the low vertebral number in anurans; somites that were initially truncal could become caudal and therefore be resorbed during metamorphosis.

In the chick, segments are lost when the tailbud regresses following a wave of apoptosis (Mills and Bellairs, 1989). An interesting finding in this context is that *Wnt-3a* is required for normal development of the tail region in mice. Homozygous null mutant *Wnt-3a*$^{-/-}$ embryos show truncation of the body at the axial level of the hindlimbs (Greco et al., 1996). These experimental data show how mutations could result in a major evolutionary change such as loss of caudal vertebrae. Other genes are important for axial elongation and tail development, notably the T-box genes including *Brachyury* (Chapman et al., 1996). Differential growth of the tailbud in the embryo can contribute to

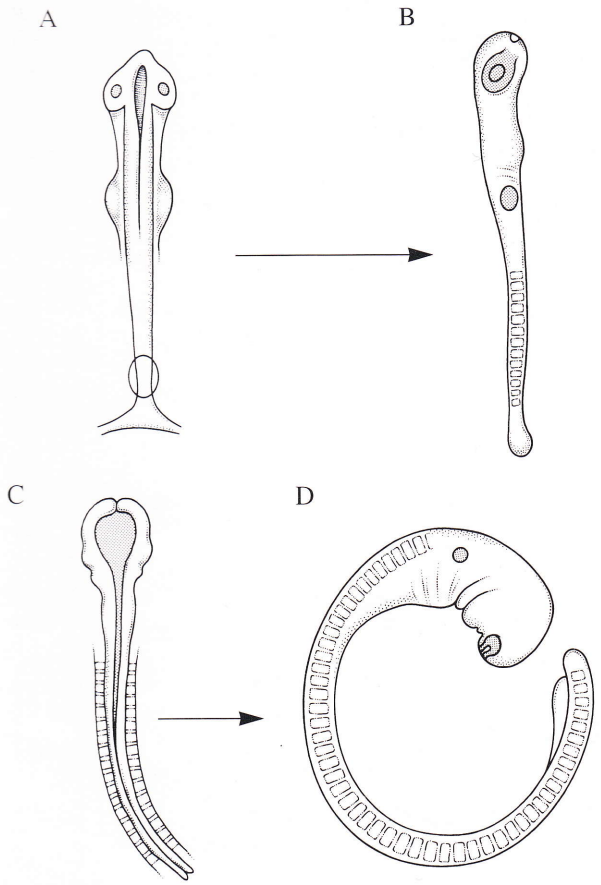

Fig. 3. Comparison of somitogenesis in a short-bodied species with few somites, and a long-bodied species with a high somite count. Illustrations based on Tavolga (1949); and Sammouri et al. (1990) with permission. (A,B) Platyfish, *Xiphophorus maculatus*. (A) Neurula stage with otic placodes and optic outgrowths. No somites have developed. Dorsal view. (B) Tailbud stage. Only 13 somites have segregated. Lateral view. (C,D) Caecilian amphibian *Typhlonectes compressicaudus*. (C) Neurula stage. In contrast to the platyfish, many somites have already appeared. Dorsal view. (D) By the tailbud stage, the body of *Typhlonectes* has become highly elongate and has over 50 somites, several times more than in the platyfish. Lateral view.

a high vertebral number in the adult. In the thresher shark *Alopias vulpinus* (Fig. 1), the tail contains nearly 300 vertebrae, more than the trunk itself (Springer and Garrick, 1964).

Snakes are a mystery because there is relatively little published data on their development. It has been suggested that the high somite number in snakes is due to the modification of phylotypic stages (Raff, 1996; Gerhart and Kirschner, 1997). In support of this view, a tailbud stage snake (*Natrix maura*) embryo has been seen with over 100 somites (A. Raynaud, unpublished). By contrast, the snake *Vipera aspis* is reported to have only 36 somites at the tailbud stage (Hubert and Dufaure, 1968), a similar number to birds or mammals. However, it is not clear whether the tailbud stage used in that study is comparable to that in other amniotes.

If snakes do have a normal somite number at the tailbud stage, compared to other amniotes, then the cloaca must slide posteriorly along the primary axis during development.

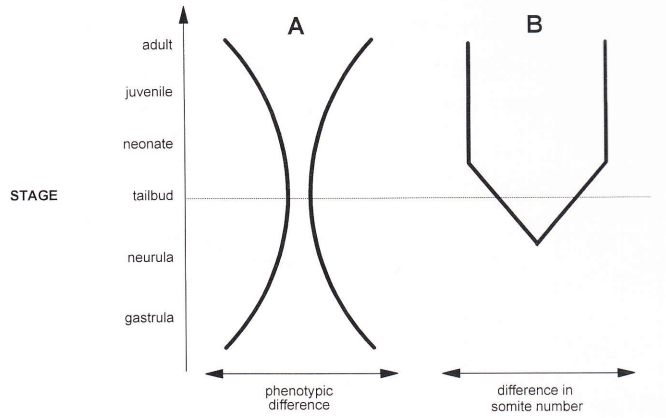

Fig. 4. Somite number in evolution and development. (A) The 'developmental hourglass' predicts that species show convergence towards, and divergence from, a conserved phylotypic (tailbud) stage. This model cannot account for differences in somite number, which show continuous divergence before, during and after the phylotypic stage. (B) A better representation of evolutionary changes in somitogenesis in this case. Based on a comparison between the platyfish and the caecilian *Typhlonectes compressicaudus* (see Fig 3). See also Richardson et al. (1997) for details of variations in somite number at the phylotypic stage.

Otherwise, adult snakes would have a short trunk and a long tail – which is not what is observed. In summary, the potential developmental targets for evolutionary changes in vertebral number include segmentation genes, tailbud growth and apoptosis, axial growth rate, somite size, vertebral fusion and cloacal displacement.

SOMITE NUMBER AND THE DEVELOPMENTAL HOURGLASS

Since somite number is a key variable in vertebrate evolution, it is important to determine which developmental stages are targets for this change. A widely accepted model of the relationship between developmental stage and phenotypic divergence is the 'developmental hourglass' or 'phylotypic egg timer' (Elinson, 1987; Wolpert, 1991; Duboule, 1994; Collins, 1995, Raff, 1996; Gerhart and Kirschner, 1997).

The hourglass model predicts that adult differences between species arise through divergence from a conserved (phylotypic) stage of embryonic development. For many anatomical characters this is clearly true, since embryos of different species often look far more alike than their respective adults. The phylotypic stage is thought to be the tailbud stage (Slack et al., 1993). There can be difficulties with identifying the tailbud stage in different species (Richardson et al., 1997). The tailbud is a compact, ectoderm-covered swelling containing mesoderm, the caudal end of the notochord and neural tube, and part of the primitive gut. When the tailbud is a distinct projection, but is not yet segmented, the tailbud stage has been reached.

'Phylotypic' stage indicates that embryos all show the body plan characteristic of their phylum, or comparable higher taxon. However, recent studies suggest that the vertebrate tailbud stage may not be as highly conserved as originally thought (Richardson, 1995; Richardson, et al., 1997). Variations in developmental timing (heterochrony) mean that the various parts

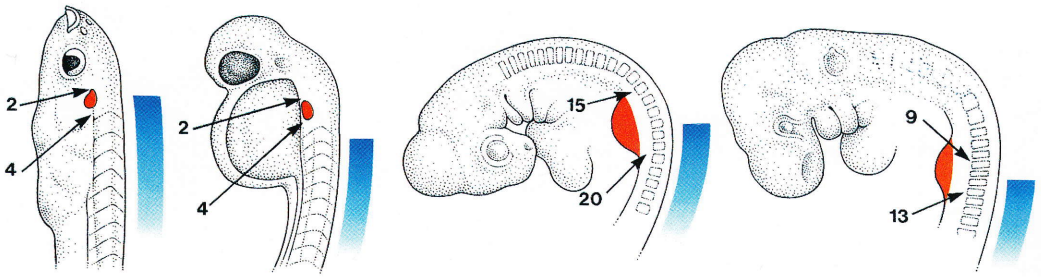

Fig. 5. Independent variation (dissociation) between somitogenesis and *Hox* gene expression (based on Burke et al., 1995, with permission). Left to right: *Xenopus*, zebrafish, chicken and mouse embryos or larvae at the limb/paired fin bud stage. The anterior expression boundary of *Hoxc-6* (blue) in the paraxial mesoderm of the embryo coincides approximately with the axial level of the forelimb or pectoral fin, even though this lies opposite different numbered somites in different species (numbered arrows). Forelimb or pectoral fin bud shown in red.

of the body plan develop at different times in different species (Richardson, 1995). For example, zebrafish embryos have no branchial arches at the tailbud stage, and the heart primordia have not fused into a tube (Richardson et al., 1997). Thus if all one knew about the zebrafish body plan came from the tailbud stage, this species would not even be placed in the same phylum as vertebrates.

Differences in somite number between some species violate the developmental hourglass

It is important to determine precisely which features of development are highly conserved and which can vary. Somitogenesis is a useful character to examine in this context because it can be easily quantified. According to the hourglass model, one would expect somite number to be similar in all species of vertebrates at a conserved stage. Adult differences in segment number should then become apparent through divergence at later stages. But this is not the case in the species examined here. The short-bodied platyfish *Xiphophorus maculatus* develops a total of 26 body segments (Tavolga, 1949). By contrast the caecilian amphibian *Typhlonectes compressicaudus* has a very long body and 98-100 segments (Sammouri et al., 1990). The development of these differences does not obey the hourglass model.

In the platyfish, somitogenesis is retarded from the earliest stages of development. At late neurula stages (Fig. 3A), no somites have developed at all and only 12-13 somites have formed by the tailbud stage (Fig. 3B). By contrast the worm-like caecilian (Amphibia: Gymnophiona) develops from an embryo which has an elongated primary axis and accelerated somitogenesis. This extreme elongation of the body is seen at the late neurula stage (Fig. 3C). Even at this early stage, 17 somites have segregated. By the tailbud stage, the primary axis of the caecilian is so elongated that it reaches right round the large egg. Over 50 somites have segregated by the tailbud stage (Fig. 3D), many more than in the platyfish at the same stage.

Thus somitogenesis appears to show an earlier *onset* and increased *rate* in the caecilian. With respect to somite number and body length, divergence between these two species begins before the tailbud stage and continues throughout it. This is in contrast to the predictions of the developmental hourglass (Fig. 4). These two species do not share a common, conserved embryonic stage that has the same, or even similar, number of somites.

Raynaud (1994) reached the same conclusion. At the tailbud stage the green lizard has 27 somites, while the slow worm has 60 and is almost twice as long. These emerging differences in body form are apparent from the earliest stages of somite segregation and cannot therefore be attributed to divergence from a shared, conserved stage. In summary, changes in somite number during evolution can, in some species, be attributed to patterns of development showing divergence beginning before the putative phylotypic stage and continuing to diverge through this and subsequent stages. It is important to realise that one does not have to use the tailbud stage as the yard stick for showing differences in somitogenesis between species. Richardson (1995) showed that a variety of developmental characters, plotted against somite number in different species, showed dissociation from somitogenesis.

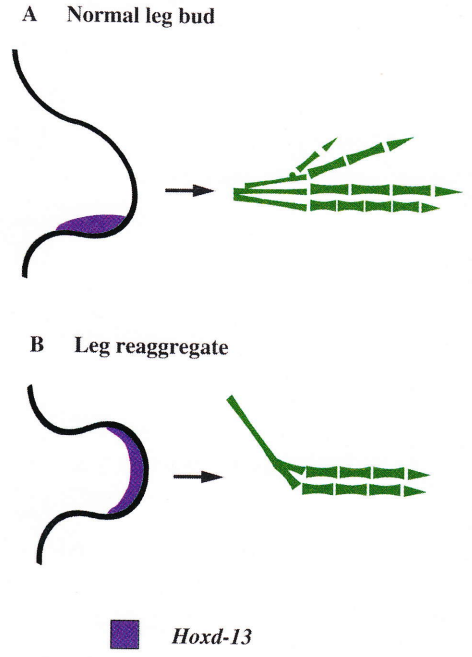

Fig. 6. Reaggregate limbs provide experimental evidence of dissociation between repeating patterns and the *Hox* genes, which encode positional value. Based on Hardy et al. (1995). (A) The normal chick leg bud (left) gives rise to a polarised digit pattern (right). Anterior is to the top, distal is to the right. *Hoxd-13* (Purple) is expressed near the posterior margin at early stages. (B) Reaggregate limb bud (left). Because the polarising region has been scrambled, *Hoxd-13* expression is not polarised. A pattern of digits is still generated, despite the lack of an AP gradient of positional information.

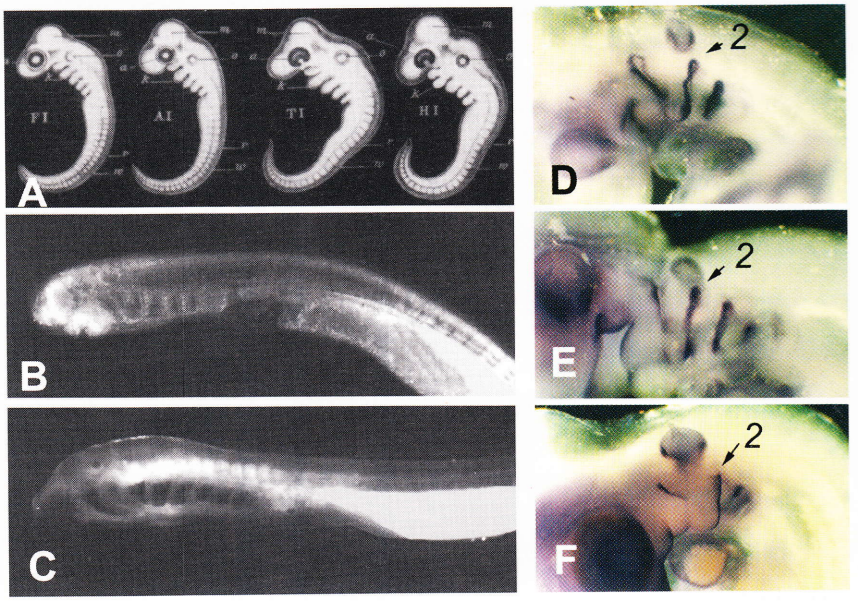

Fig. 7. Evolutionary dissociation between segmentation and positional specification in the pharyngeal apparatus. (A) Haeckel's (1874) drawing of a universal embryonic stage for vertebrates. From left to right: 'fish', urodele, turtle, chick. (B,C) Lamprey *(Petromyzon marinus)* at 12 and 21 days, respectively (specimens from the laboratory of G. M. Wright). These embryos do show the simple series of pharyngeal arches shown by Haeckel (1874). (D-F) Chick embryo pharyngeal region showing that Haeckel's (1874) stylised series of pharyngeal pouches cannot be recognised. To demonstrate pharyngeal development, we have used a chick *bmp-7* probe (Houston et al., 1994). *bmp-7* is a marker for the pharyngeal cleft ectoderm (Wall and Hogan, 1995). At HH stage 16 (D), pharyngeal clefts 1-3 are distinct. At stage 19 (E), the 1st cleft is becoming remodelled and the 4th cleft is appearing. By stage 24 (F), the 1st cleft is almost obliterated and the caudalmost clefts are only just becoming visible. Therefore, no complete – but undifferentiated – pharyngeal series is seen at any one stage in the chick. This is in contrast to the images in many textbooks. 2 and arrow, 2nd pharyngeal cleft.

SOMITE NUMBER AND THE ZOOTYPE

We now consider how it is possible for somite number to vary so widely between species at the tailbud stage – the putative phylotypic stage – when this stage is thought to be constrained by conserved patterns of developmental gene expression.

The zootype and evolutionary constraint

A key advance in evolutionary developmental biology has been the identification of the zootype – a conserved pattern of homeobox gene expression seen in a wide range of animal taxa (Slack et al., 1993; Manak and Scott, 1994). These genes may encode positional value along the primary axis. The existence of the zootype is consistent with Wolpert's concept of a universal positional field (Wolpert, 1989). Zootypic genes are thought to be expressed most strongly at phylotypic (tailbud) stages. However, recent work has shown that phylotypic stages are more variable than is widely assumed (Richardson, 1995; Richardson et al., 1997). Furthermore, the variations in somite number at the phylotypic stage described above are not consistent with evolutionary constraint in this feature. Changes in phylotypic stages are perhaps to be expected; the zootype has itself evolved – by gene duplication and loss.

Raff (1996) has identified two opposing positions in evolutionary developmental biology, one favouring developmental conservatism and the other stressing the evolutionary lability of embryonic stages. We suggest that, at least with respect to certain features such as somitogenesis, these conflicting views are easily reconciled: the body plan consists of some structures that are dependent on specific *Hox* codes for their determination and are therefore highly constrained, and others that show *dissociation* or uncoupling from the zootype and are therefore more easily modified during evolution. We use the term dissociation (uncoupling, disengagement or dissociability; Needham, 1950; Richardson, 1995) to imply that two mechanisms, acting on the same pattern, can be varied independently during evolution.

Examples of genes that may encode specific structures, rather than relative position, include the non-zootype homeobox genes *tinman* and *Pax6*. These may specify heart and eye, respectively (Azpiazu and Frasch, 1993; Scott, 1994; Schultheiss et al., 1995). *tinman* is expressed in cardiac precursors in *Drosophila* and vertebrates, even though the hearts of these animals are not traditionally considered homologous. Specific structures can also be encoded by zootypic genes. For example *orthodenticle* homologues encode for the rostral neural tube region and vision-associated cells in a range of chordates (Williams and Holland, 1996). The position of the forelimb and associated structures is also specified by characteristic *Hox* codes (Gaunt, 1994; Burke et al., 1995).

Somite number is uncoupled from the zootype

Somite identity is controlled independently of somite number (Cooke, 1975). Cooke predicted that there were unlikely to be genes specifying the identity of a particular numbered somite. This is now thought to be true. It is likely that axial identity along the vertebral column is specified according to a *Hox* code (Kessel and Gruss, 1991). Treatment of embryos with retinoic acid causes an anterior shift both in the boundaries of *Hox* gene expression, and in the axial identity of vertebrae (Kessel and Gruss, 1991). Experimental transpositions* of this type support the view that segment identity can be varied independently of segment number.

Transpositions are also seen during evolution. In a landmark paper, Burke and colleagues compared the axial level of *Hoxc-6* expression in species with different vertebral formulae (Burke et al., 1995). The anterior boundary of *Hoxc-6* expression in the paraxial mesoderm lay opposite different somite numbers in different species (Fig. 5). Yet it always corresponded to the axial level of the brachial plexus, even though this varies widely between species (Fig. 5). Thus evolutionary changes in the axial level of the forelimb are associated with a corresponding shift in the axial level of *Hox*

*Shifts in axial level characterisitcs.

gene expression. Gaunt (1994) reached similar conclusions. These studies indicate that the specification of positional values in the mesoderm is independent of the specification of boundaries between somites. Therefore, because somitogenesis is not tied to particular *Hox* codes, it is freed from the evolutionary constraint of the zootype.

OTHER EXAMPLES OF DISSOCIATION

Somitogenesis can be uncoupled from the zootype. We need to know if this is an isolated phenomenon or part of a wider pattern in vertebrate evolution. We argue that there are other examples of repeating patterns uncoupled from the genes that encode segment identity.

Dissociation in limb development

Early models of limb development proposed that a morphogen gradient specified not only the pattern of digits, but also digit identity (Tickle et al., 1975). Thus a single mechanism accounted for the whole pattern and no dissociation was possible. But it later became apparent that there may be two distinct mechanisms that interact to give the final pattern: a repeating pattern to generate a ground plan of skeletal elements and a positional field to make the digits non-equivalent (Wolpert and Stein, 1984; Wolpert, 1989; Tabin, 1992).

The two mechanisms can be uncoupled experimentally in reaggregates. These are made by placing a pellet, reaggregated from a single-cell suspension of limb-bud mesenchyme, into an ectodermal limb-bud jacket. When grafted to a host, the reaggregates give rise typically to 1-3 identical digit-like structures (Ros et al., 1994; Hardy et al., 1995). When *Hoxd* gene expression was examined in the reaggregate, a uniform pattern across the digits was seen, instead of a series of nested domains (Ros et al., 1994; Hardy et al., 1995). Thus a pattern of digits had been generated in the absence of a gradient of positional information (Fig. 6).

Feather patterns

Like the somites and the limb, feather patterns (and the associated feather pigment patterns) probably involve a repeating pattern and a mechanism for specifying positional information (Richardson et al., 1989, 1990, 1991). Experiments suggest that feathers are laid down according to a spacing mechanism (McLachlan, 1980; Davidson, 1983). A separate mechanism makes the feather germs different from one another.

Feather number may evolve independently of the positional field that determines feather identity. Thus although the same types of feathers (small covets or large flight feathers for example) may be present in different species, the number of rows of feathers of each type can vary between species (Lucas and Stettenheim, 1972). This could be achieved by a change in the spacing frequency of the feathers, or the size of the pteryla, with no need to invoke changes in *Hox* gene regulation. Again, because of dissociation, patterns can vary even though one mechanism involved in their specification is a conserved positional field.

The hindbrain and pharyngeal arches

In the vertebrate hindbrain, *Hox* genes may provide a positional field, which is superimposed upon a segmental ground plan (Wilkinson et al., 1989; Zhang et al., 1994). Studies on the murine segmentation mutant *kreisler* suggest that *Hox* codes can be established independently of segmentation (Frohman et al., 1993). This view is supported by the fact that retinoic acid has different effects on segmentation and the specification of segment identity in the hindbrain (Wood and Thorogood, 1994).

However, there is also evidence that hindbrain segmentation and positional specification are not independent mechanisms (Krumlauf, 1992; Lumsden and Krumlauf, 1996). Both *Krox-20* and *kreisler/valentino* probably regulate *Hox* gene expression, suggesting that they are related to the control of segment identity as well as to segmentation itself (Sham et al., 1993; Moens et al., 1996). This interdependence of functions might explain why rhombomere number is far more stable during evolution than somite number. Another factor may be that rhombomere segmentation lacks the open-ended properties of somitogenesis in the tailbud.

The pattern of *Hox* genes expressed in the hindbrain appears to specify positional values of cells in the pharyngeal arches (Hunt and Krumlauf, 1992). We believe that there is good evidence of independent variation of segmentation and positional specification in the pharyngeal apparatus. In lampreys, the simple, segmental pattern of pharyngeal pouches laid down in the embryo remains largely unaltered through later stages and the embryo therefore resembles the adult (Fig. 7B,C). By contrast, in the chicken and other amniote embryos, expression patterns of *bmp-7* reveal that the anterior pharyngeal clefts have become remodelled before the posterior ones have even formed (Fig. 7D-F). Because of this, chick embryos never display the complete, undifferentiated series of arches depicted by Haeckel (1874).

CONCLUSIONS

Variations in somite number suggest to us that patterns of homeobox gene expression do not always impose rigid evolutionary constraint on embryonic development. Homeobox genes appear to function in two distinct ways. They can encode non-repeating structures such as eyes or heart; or they can encode position value in a series of repeating elements. In the latter case, the final pattern is determined by two distinct mechanisms. If these are uncoupled from each other, as they appear to be in the development of somites, limb, feathers and pharyngeal arches, the system can show considerable evolutionary lability. Thus the body plan consists of different components with different degrees of evolutionary constraint. It is possible that at least some of the highly constrained components correspond to Cooke's (1975) non-repeating elements.

It is widely recognised that all vertebrate embryos share a common and highly conserved developmental programme. However, dissociation of developmental mechanisms is at odds with the widespread view that certain embryonic stages remain almost unchanged during evolution (Haeckel, 1874; Elinson, 1987; Wolpert, 1991; Duboule, 1994; Collins, 1995, Gerhart and Kirschner, 1997). Some authors have suggested that developmental mechanisms in embryos are so tightly coupled that any evolutionary change in embryonic development is

likely to be lethal (Collins, 1995). We believe that, although developmental mechanisms are highly conserved, dissociation allows at least some evolutionary modifications to be made, even at phylotypic stages. Modifications of this type have been important in the evolution of body form and vertebral number in several vertebrate groups.

Special thanks are due to Michael Akam for critical contributions. Many other scientists have shared ideas with us, including Mike Coates, Ron Conlon, Jonathan Cooke, Jacqueline Deschamps, Brian Hall, Peter Holland, Kirsty Lawson, Andrew Lumsden, Alessandro Minelli, Bill Perrin, Jose de la Pompa, Sabine Renous, Jonathan Slack, Claudio Stern and David Wilkinson. Brian Houston kindly provided the chick *bmp-7* probe and Philippa Francis-West commented on the manuscript. John Bluemink and the staff of the Netherlands Institute for Developmental Biology made embryos available for study. Richard Tibbitts prepared the line drawings. Financial support was provided by the British Heart Foundation (M. K. R. and S. P. A.), The Wellcome Trust and British Council (M. K. R.), and The NSF, IBN 94-19407 (J. H.).

REFERENCES

Anderson, D. T. (1973). *Embryology and Phylogeny in Annelids and Arthropods.* Oxford: Pergamon.

Anderson, S. and Jones, J. K. (1984) *Orders and Families of Recent Mammals of the World.* New York: John Wiley and Sons.

Azpiazu, N. and Frasch, M. (1993). *tinman* and *bagpipe*: two homeo box genes that determine cell fates in the dorsal mesoderm of Drosophila. *Genes Dev.* **7,** 1325-1340.

Baer, J. G. (1964) *Comparative Anatomy of Vertebrates.* London: Butterworths.

Burke, A. C., Nelson, C. E., Morgan, B. A. and Tabin, C. (1995). *Hox* genes and the evolution of vertebrate axial morphology. *Development* **121,** 333-346.

Chapman, D. L., Agulnik, I., Hancock, S., Silver, L. M., and Papaioannou, V. E. (1996). Tbx6, a mouse T-Box gene implicated in paraxial mesoderm formation at gastrulation. *Dev. Biol.* **180,** 534-542.

Christ, B. and Wilting, J. (1992). From somites to vertebral column. *Anat. Anz.* **174,** 23-32.

Collins, P. (1995) Embryology and Development. In *Gray's Anatomy.* pp 91-341. New York: Churchill Livingstone.

Conlon, R. A., Reaume, A. G. and Rossant, J. (1995). *Notch1* is required for the coordinate segmentation of somites. *Development* **121,** 1533-1545.

Cooke, J. (1975). Control of somite number during morphogenesis of a vertebrate, *Xenopus laevis*. *Nature* **254,** 196-199.

Cooke, J. (1981). The problem of periodic patterns in embryos. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **295,** 509-524.

Davidson, D. (1983). The mechanism of feather pattern development in the chick. 1. The time of determination of feather position. *J. Embryol. Exp. Morphol.* **74,** 245-259.

de la Pompa, J. L., Wakeham, A., Correia, K. M., Samper, E., Brown, S. Aguilera, R. J., Nakano, T., Honjo, T., Mak, TW., Rossant J. and Conlon RA. (1997). Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development.* **124,** 1139-1148.

De Robertis, E. M. (1997). The ancestry of segmentation. *Nature* **387,** 25-26.

Dean, B. (1899) On the embryology of *Bdellostoma stouti*. In *Feitschrift zum siebenzigsten Geburtstag von Carl von Kupffer.* pp 221-276. Jena: Fischer-Verlag.

Duboule, D. (1994). Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Development* **1994 Supplement** 135-142.

Duellman, W. E. and Trueb, L. (1994) *Biology of Amphibians.* Baltimore: Johns Hopkins University Press.

Elinson, R. P. (1987) Changes in developmental patterns: embryos of amphibians with large eggs. In *Development as an evolutionary process.* (ed. R. A. Raff). pp 1-21. New York: Alan R. Liss.

Frohman, M. A., Martin, G. R., Cordes, S. P., Halamek, L. P. and Barsh, G. S. (1993). Altered rhombomere-specific gene expression and hyoid bone differentiation in the mouse segmentation mutant, kreisler (kr). *Development* **117,** 925-936.

Gaunt, S. J. (1994). Conservation in the Hox code during morphological evolution. *Int. J. Dev. Biol.* **38,** 549-552.

Gerhart, J. C. and Kirschner, M. W. (1997). *Cells, Embryos and Evolution.* Oxford: Blackwell.

Greco, T. L., Takada, S., Newhouse, M. M., McMahon, J. A., McMahon, A. P. and Camper, S. A. (1996). Analysis of the vestigial tail mutation demonstrates that Wnt-3a gene dosage regulates mouse axial development. *Genes Dev.* **10,** 313-324.

Gont, L. K., Steinbeisser, H., Blumberg, B. and De Robertis, E. M. (1993). Tail formation as a continuation of gastrulation: the multiple cell populations of the *Xenopus* tailbud derive from the late blastopore lip. *Development* **119,** 991-1004.

Haeckel, E. (1874) *Anthropogenie oder Entwickelungsgeschichte des Menschen.* Leipzig: Engelmann.

Hardy, A., Richardson, M. K., Francis-West, P. H., Rodriguez, C., Izpisua-Belmonte, JC, Duprez, D. and Wolpert, L. (1995). Gene expression, polarising activity and skeletal patterning in reaggregated hind limb mesenchyme. *Development* **121,** 4329-4337.

Holland, L. Z., Kene, M., Williams, N. A. and Holland, N. D. (1997). Sequence and embryonic expression of the amphioxus *engrailed* gene *(AmphiEn)*: the metameric pattern of transcription resembles that of its segment-polarity homolog in *Drosophila*. *Development* **124,** 1723-1732.

Holland, N. D., Holland, L. Z., Honma, Y. and Fujii, T. (1993). *Engrailed* expression during development of a lamprey, *Lampetra japonica*: a possible clue to homologies between agnathan and gnathostome muscles of the mandibular arch. *Dev. Growth. Differen.* **35,** 153-160.

Houston, B., Thorp, B. H. and Burt, D. W. (1994). Molecular cloning and expression of bone morphogenetic protein-7 in the chick epiphyseal growth plate. *J. Mol. Endocrinol.* **13,** 289-301.

Hrabe de Angelis, M., McIntyre, J. and Gossler, A. (1997). Maintenance of somite borders in mice requires the *delta* homologue *Dll1*. *Nature* **386,** 717-721.

Hubert, J. and Dufaure, J. P. (1968). Table de développement de la vipère aspic, *Vipera aspis* L. *Bull. Soc. Zool. France* **93**, 135-148.

Hunt, P. and Krumlauf, R. (1992). Hox codes and positional specification in vertebrate embryonic axes. *Annu. Rev. Cell Biol.* **8,** 227-256.

Ingham, P. W. and Martinez Arias, A. (1992). Boundaries and fields in early embryos. *Cell* **68,** 221-235.

Irving, C., Nieto, M. A., DasGupta, R., Charnay, P. and Wilkinson, D. G. (1996). Progressive spatial restriction of Sek-1 and Krox-20 gene expression during hindbrain segmentation. *Dev. Biol.* **173,** 26-38.

Kessel, M. and Gruss, P. (1991). Homeotic transformations of murine vertebrrae and concomitant alterations of *flox* codes induced by retinoic acid. *Cell* **67**, 89-104.

Keynes, R. and Stern, C. (1988). Mechanisms of segmentation in vertebrates. *Development* **103,** 413-429.

Kimmel, C. B., Sepich, D. S. and Trevarrow, B. (1988). Development of segmentation in zebrafish. *Development* **104 Supplement,** 197-207.

Krumlauf, R. (1992). Evolution of the vertebrate Hox homeobox genes. *BioEssays* **14,** 245-252.

Lawrence, P. A. (1992) *The Making of a Fly: the Genetics of Animal Design.* Oxford: Blackwell.

Lucas, A. M. and Stettenheim, P. R. (1972). *Avian Anatomy: Integument.* Part 1. Washington DC: United States Dept. Agriculture.

Lumsden, A. and Krumlauf, R. (1996). Patterning the vertebrate neuraxis. *Science* **274,** 1109-1115.

Manak, J. R. and Scott, M. P. (1994). A class act: conservation of homeodomain protein functions. *Development* **1994 Supplement,** 61-77.

McLachlan, J. C. (1980). The effect of 6-aminonicotinamide on limb development. *J. Embryol. Exp. Morphol.* **55,** 307-318.

Mills, C. L. and Bellairs, R. (1989). Mitosis and cell death in the tail of the chick embryo. *Anat. Embryol. (Berl.)* **180,** 301-308.

Minelli, A. (1992). Towards a new comparative morphology of myriapods. *Ber. nat.-med. Ver. Innsbruck* Suppl. **10**, 37-46.

Moens, C. B., Yan, Y. L., Appel, B., Force, A. G. and Kimmel, C. B. (1996). *valentino*: a zebrafish gene required for normal hindbrain segmentation. *Development* **122,** 3981-3990.

Müller, M. v. Weizsacker, E. and Campos-Ortega, J. A. (1996). Expression domains of a zebrafish homologue of the *Drosophila* pair-rule gene *hairy* correspond to primordia of alternating somites. *Development* **122,** 2071-2078.

Nagy, L. M. and Carroll, S. (1994). Conservation of *wingless* patterning functions in the short-germ embryos of *Tribolium castaneum*. *Nature*, **367,** 460-463.

Needham, J. (1950). *Biochemistry and Morphogenesis*. Cambridge University Press.

Nieto, M. A., Gilardi-Hebenstreit, P., Charnay, P. and Wilkinson, D. G. (1992). A receptor protein tyrosine kinase implicated in the segmental patterning of the hindbrain and mesoderm. *Development* **116,** 1137-1150.

Nieuwkoop, P. D. and Faber, J. (1967) *Normal Table of Xenopus laevis.* Amsterdam: North-Holland.

Pankratz, M. J. and Jackle, H. (1993). Blastoderm Segmentation. In *The Development of Drosophila melanogaster* (ed. M. Bate, A. Martinez-Arias). pp. 467-516. Vol. 1. New York: Cold Spring Harbor

Primmett, D. R., Norris, W. E., Carlson, G. J., Keynes, R. J. and Stern, C. D. (1989). Periodic segmental anomalies induced by heat shock in the chick embryo are associated with the cell cycle. *Development* **105,** 119-130.

Primmett, D. R., Stern, C. D. and Keynes, R. J. (1988). Heat shock causes repeated segmental anomalies in the chick embryo. *Development* **104,** 331-339.

Raff, R. A. (1996) *The Shape of Life; Genes, Development and the Eevolution of Animal Form.* Chicago: University of Chicago Press.

Raynaud, A. (1994). Données préliminaires sur l'allongement du corps et la somitogenèse chez les jeunes embryons d'Orvet (*Anguis fragilis,* L.) et de lézard vert (*Lacerta viridis,* Laur.). *Bull. Soc. Hist. Nat. Toulouse,* **130,** 47-52.

Richardson, M. K., Hornbruch, A. and Wolpert, L. (1989). Pigment pattern expression in the plumage of the quail embryo and the quail-chick chimaera. *Development* **107**, 805-818.

Richardson, M. K., Hornbruch, A. and Wolpert, L. (1990). Mechanisms of pigment pattern formation in the quail embryo. *Development* **109,** 81-89.

Richardson, M. K., Hornbruch, A. and Wolpert, L. (1991). Pigment patterns in neural-crest chimeras constructed from quail and guinea fowl embryos. *Dev. Biol.* **143,** 309-319.

Richardson, M. K. (1995). Heterochrony and the phylotypic period. *Dev. Biol.* **172,** 412-421.

Richardson, M. K., Hanken, J., Gooneratne, M. L., Pieau, C., Raynaud, A., Selwood, L. and Wright, G. M. (1997). There is no highly conserved stage in vertebrate embryonic development: implications for current theories of evolution and development. *Anat. Embryol.* **196,** 91-106.

Ros, M. A., Lyons, G. E., Mackem, S. and Fallon, J. F. (1994). Recombinant limbs as a model to study homeobox gene regulation during limb development. *Dev. Biol.* **166,** 59-72.

Sammouri, R., Renous, S., Exbrayat, J. M. and Lescure, J. (1990). Développement embryonnaire de *Typhlonectes compressicaudus* (Amphibia, Gymnophiona). *Ann. des Sci. Nat. Zool. Paris* **11,** 135-163.

Schneider-Maunoury, S., Seitanidou, T., Charnay, P. and Lumsden, A. (1997). Segmental and neuronal architecture of the hindbrain of Krox-20 mouse mutants. *Development* **124,** 1215-1226.

Schultheiss, T. M., Xydas, S. and Lassar, A. B. (1995). Induction of avian cardiac myogenesis by anterior endoderm. *Development* **121,** 4203-4214.

Scott, M. P. (1994). Intimations of a creature. *Cell* **79,** 1121-1124.

Selleck, M. A. and Stern, C. D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development* **112,** 615-626.

Sham, M. H., Vesque, C., Nonchev, S., Marshall, H., Frain, M., Gupta, R. D., Whiting, J, Wilkinson, D., Charnay, P. and Krumlauf, R. (1993). The zinc finger gene Krox20 regulates HoxB2 (Hox2. 8) during hindbrain segmentation. *Cell* **72,** 183-196.

Slack, J. M., Holland, P. W. and Graham, C. F. (1993). The zootype and the phylotypic stage. *Nature* **361,** 490-492.

Springer, V. G. and Garrick, J. A. F. (1964). A survey of vertebral numbers in sharks. *Proc United States Nat. Mus.* **116,** 73-96.

Starck, J. M. (1996). Comparative morphology and cytokinetics of skeletal growth in hatchlings of altricial and precocial birds. *Zool. Anz.* **235,** 53-75.

Stern, C. D., Fraser, S. E., Keynes, R. J. and Primmett, D. R. (1988). A cell lineage analysis of segmentation in the chick embryo. *Development* **104 Supplement,** 231-244.

Stern, C. D., Hatada, Y., Selleck, M. A. J. and Storey, K. G. (1992). Relationships between mesoderm induction and the embryonic axes in chick and frog embryos. *Development* **1992 Supplement,** 151-156.

Summerbell, D., Lewis, J. H. and Wolpert, L. (1975). Positional information in chick limb morphogenesis. *Nature* **244,** 492-496.

Tabin, C. J. (1992). Why we have (only) five fingers per hand: *hox* genes and the evolution of paired limbs. *Development.* **116,** 289-296.

Tam, P. P. (1981). The control of somitogenesis in mouse embryos. *J. Embryol. Exp. Morphol.* **65 Supplement** 103-128.

Tavolga, W. N. (1949). Embryonic development of the Platyfish *(Platypoecilus),* the swordtail *(Xiphophorus)* and their hybrids. *Bull. Am. Mus. Nat. Hist.* **94,** 165-229.

Tickle, C., Summerbell, D. and Wolpert, L. (1975). Positional signalling and the specification of digits in chick limb morphogenesis. *Nature* **254**, 199-202.

Townsend, D. S. and Stewart, M. M. (1985). Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): a staging table. *Copeia* **1985,** 423-436.

Tucker, A. S. and Slack, J. M. W. (1995). The *Xenopus laevis* tail-forming region. *Development* **121,** 249-262.

Verbout, A. J. (1985). The development of the vertebral column. *Adv. Anat. Embryol. Cell Biol.* **90,** 1-122.

Wall, N. A. and Hogan, B. L. (1995). Expression of bone morphogenetic protein-4 (BMP-4), bone morphogenetic protein-7 (BMP-7), fibroblast growth factor-8 (FGF-8) and sonic hedgehog (SHH) during branchial arch development in the chick. *Mech. Dev.* **53,** 383-392.

Weisblat, D. A., Price, D. J. and Wedeen, C. J. (1988). Segmentation in leech development. *Development* **104 Supplement,** 161-168.

Wilkinson, D. G., Bhatt, S., Cook, M., Boncinelli, E. and Krumlauf, R. (1989). Segmental expression of Hox-2 homoeobox-containing genes in the developing mouse hindbrain. *Nature* **341,** 405-409.

Williams, N. A. and Holland, P. W. H. (1996). Old head on young shoulders. *Nature* **383,** 490.

Wolpert, L. (1989). Positional information revisited. *Development* **1989 Supplement,** 3-12.

Wolpert, L. (1991). *The Triumph of The Embryo*. Oxford: Oxford University Press.

Wolpert, L. and Stein, W. D. (1984) Positional information and pattern formation. In *Pattern Formation.* (ed. G. M. Malacinski and S. V. Bryant). pp 2-21. New York: MacMillan.

Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J. S., Trumbauer, M. E., Chen, H. Y., Price, D. L., Van der Ploeg, L. H. T. and Sisodia, S. S. (1997). Presenilin 1 is required for *Notch1* and *Dll1* expression in the paraxial mesoderm. *Nature* **387,** 288-292.

Wood, A. and Thorogood, P. (1994). Patterns of cell behaviour underlying somitogenesis and notochord formation in intact vertebrate embryos. *Dev. Dyn.* **201,** 151-167.

Xu, Q., Alldus, G., Holder, N. and Wilkinson, D. G. (1995). Expression of truncated *Sek-1* receptor tyrosine kinase disrupts the segmental restriction of gene expression in the *Xenopus* and zebrafish hindbrain. *Development* **121,** 4005-4016.

Young, J. Z. (1962). *The Life of Vertebrates.* London: Oxford University Press.

Zhang, M., Kim, H. J., Marshall, H., Gendron-Maguire, M., Lucas, D. A., Baron, A., Gudas, L. J., Gridley, T., Krumlauf, R. and Grippo, J. F. (1994). Ectopic *Hoxa-1* induces rhombomere transformation in mouse hindbrain. *Development* **120,** 2431-2442.